AD_____

Award Number: DAMD17-02-1-0389

TITLE: CRE Activation in Antiestrogen Resistance

PRINCIPAL INVESTIGATOR: Bianca P. Gomez
Robert Clarke, Ph.D.

CONTRACTING ORGANIZATION: Georgetown University
Washington, DC 20007

REPORT DATE: May 2003

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	May 2003	Annual Summary (1 May 2002 - 30 Apr 2003)

4. TITLE AND SUBTITLE
CRE Activation in Antiestrogen Resistance

5. FUNDING NUMBERS
DAMD17-02-1-0389

6. AUTHOR(S)
Bianca P. Gomez
Robert Clarke, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Georgetown University
Washington, DC 20007

E-Mail: bs1294tgo@aol.com

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

The over-expression of the human X-base binding protein (h-XBP-1) and hence elevated levels of CRE transcription activator contributes to the antiestrogen resistance. Preliminary data using Western blot analysis indicates that E2 –independent but responsive breast cancer cells that are responsive to antiestrogen showed elevated hXBP-1 protein level whereas, the cell line that is resistant to antiestrogen showed a lower level of hXBP-1 protein expression. Furthermore, a promoter- reporter assay measuring luciferase activity as an indicator of CRE activation showed that cells that are antiestrogen resistant had a four- fold increase in CRE activation when compared to the sensitive cells. HXBP-1 is transcription factor that belongs to the ATF/CRE family of transcription factors and can bind to and activate the CREs (cyclic AMP response elements). It is based on this relationship that the amount of hXBP-1 protein levels could be determined by measuring the level of CRE containing genes. In our experiments by over expressing hXBP-1 using transfection we well convert sensitive cells to resistant cell. On the other hand, cell resistant to antiestrogen will become sensitive by using an antisense oligonucleotide approach. Anchorage dependent as well as anchorage independent growth responses of the respective transformed cells to antiestrogen will also be measured.

14. SUBJECT TERMS
HXBP-1-Human X-box binding protein; CRE- cyclic AMP response elements; E2-Estrogen

15. NUMBER OF PAGES
10

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

___ Where copyrighted material is quoted, permission has been obtained to use such material.

___ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

___ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

__ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

__ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

N/A In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

07·02·03 _____ PI - Signature

Date

Table of Contents

Cover.....1

SF 298.....2

Foreword.....3

Table of Contents.....4

Introduction.....5

Body.....6

Key Research Accomplishments.....7

Reportable Outcomes.....8

Conclusions.....9

References.....10

Appendices.....

INTRODUCTION

Human X box bonding protein (hXBP-1) has been associated with antiestrogen resistance in our lab. We believe that by the up regulation of hXBP-1 due to the over-expression of this gene, that there is some contribution to antiestrogen resistance. This resistance to antiestrogens may occur by growth induction or by apoptosis blockage. Once we have up regulated hXBP-1 it is expected that there must be increased CRE transcriptional activation, this will be measured by promoter- reporter assays. The purpose of these experiments is based on the fact that most patients with initial responsive breast tumors to antiestrogens acquire antiestrogen resistance (1). Although several possible mechanisms such as mutations in the estrogen receptors (ER) and loss of the ER expression have been associated with antiestrogen resistance other possible mechanisms that can be considered to contribute to antiestrogen resistance may also include Tamoxifen stimulated resistance mechanisms (1). Tamoxifen (TAM) or ICI 46, 477 is the most widely used antiestrogen for breast cancer treatment. Faslodex (ICI 182, 780) is the other antiestrogen therapy used for breast cancer treatment (2). It is effective in patients who have acquired resistance to TAM treatment. In our experiments the two cell lines MCF7 and T 47D are being used to determine resistance to these antiestrogens when hXBP-1 is overexpressed.

BODY

1. MCF7 cells sensitive to antistrogen were transfected with a pcDNA 3.1 vector (Invitrogen) containing a hXBP-1 insert for hXBP-1 over-expression. The empty vector was also transfected into MCF-7 cells to create a negative control.

2. Clone resistant to G 418 were selected to determine clones positive for the pcDNA 3.1/ hXBP-1 insert. Clones were expanded using 6 well plates.

3. hXBP-1 protein was extracted from these clones using Trizol reagent (Invitrogen). The amounts of proteins expressed in these cells were determined by Western blot analysis.

4. HXBP-1 activity was determined by measuring CRE transcriptional activation using a dual luciferase promoter- reporter assay system (Promega).

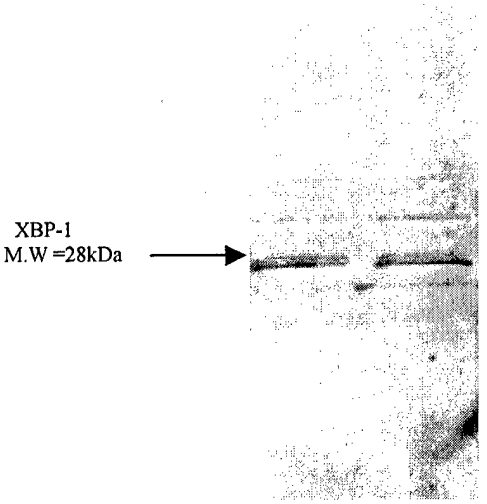

Fig. 1. Western blot to show two clones harboring hxbp-1 insert.

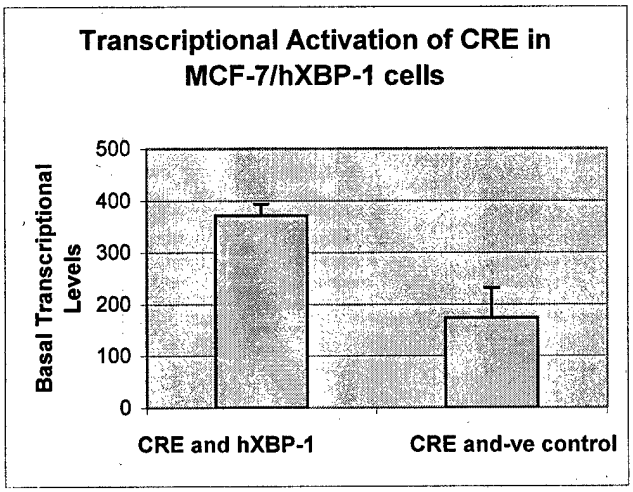

Fig.2. Transcriptional activation of hXBP-1 and CRE in MCF-7 cells. Negative control is empty pCDNA3.1 vector

KEY RESEARCH ACCOMPLISHMENTS

Task 1. To determine if sensitive cells can become resistant to antiestrogens Tamoxifen and Faslodex by the over-expression of hXBP-1 in breast cancer cells (Months 1-12):

- Transfected hXBP-1 into MCF/LCC1 (sensitive cells) with the vector containing the hXBP-1 insert.
- Cells were plated in media containing G418 to select resistant colonies (Months 1-6).
- Measured hXBP-1 expression levels in selected clones (Months 6-10).
- Confirmed hXBP-1 activity by measuring CRE transcriptional activity (Months 10-12).
- Currently T47D cells transfected with the pCDNA3.1 vector containing the hXBP-1 insert as well as the empty vector used as the negative control are now been grown in selective media for the selection of positive clones.

Course taken during the academic year 2001-2002: TBIO/PBIO 595 Tumor Endocrinology (3 credits)

REPORTABLE OUTCOMES

None at this current time.

CONCLUSION

- Over-expression of hXBP-1 was accomplished by the transfection of MCF-7 cells.

- Elevated levels of CRE in MCF7 cells transfected with the hXBP-1 indicate up regulation of hXBP-1.

- The MCF-7/hXBP-1 cells are now available for further characterization to explain the effects of XBP-1 expression on hormone responsiveness.

REFERENCES

1. Clarke,R., Leonessa, F., Welch, J.N., and Skaar, T.C.Cellular and Molecular pharmacology of antiestrotgen action and resistance. Pharmacol Rev, 53:25-71,2001.

2. Howell, A.,DeFriend, D., Robertson, J.F.R., Blamey, R.W., and Walton, P. Responses to a specific antiestrogen (ICI 182,780) in tamoxifen-resistant breast cancer. Lancet, 345: 29-30, 1995.